

Characterization of a New Enterococcal Gene, *satG*, Encoding a Putative Acetyltransferase Conferring Resistance to Streptogramin A Compounds

Streptogramin antibiotics are mixtures of two chemically unrelated A and B compounds that act synergistically in vivo against gram-positive pathogens, such as staphylococci, streptococci, and enterococci (8, 11). Resistance against B compounds is very widespread among enterococci and is mediated via the *ermB* gene cluster (e.g., on Tn917) that confers macrolide-lincosamide-streptogramin B resistance (7). The synergistic mixture of streptogramins A and B overcomes resistance to B compounds but is inactive in resistance to A compounds. The only known resistance mechanism against streptogramin A compounds in enterococci is mediated by the streptogramin acetyltransferase SatA (9). *Enterococcus faecium* isolates with *satA*-mediated resistance have been found in samples of human and animal origins, indicating a possible spread of resistance genes or resistant bacteria among different ecosystems (10).

We isolated a quinupristin-dalfopristin-resistant *E. faecium* UW1965 from a sewage treatment plant in Germany. The resistance determinant was transferred to a susceptible recipient, producing the transconjugant UW1965K1. UW1965K1 is resistant to quinupristin-dalfopristin (MIC ≥ 16 μ g/ml) and virginiamycin M (A compound; MIC, 16 μ g/ml), whereas the

MIC of each antibiotic for the recipient was 1 μ g/ml. PCR amplification for the *satA* gene was negative.

In staphylococci, resistance to streptogramin A compounds is mediated by two mechanisms: (i) acetylation of the streptogramin A via acetyltransferases (Vat, VatB, and VatC [1-3]) and (ii) efflux due to an ABC transporter (Vga and VgaB [4, 5]). PCR amplification for the *vat*, *vatB*, *vatC*, and *vga* genes failed to produce any product. The putative protein sequences of the known streptogramin acetyltransferases in staphylococci and enterococci contain three conserved motifs (2). Corresponding primers, *satI* and *satJ*, have been made, producing a 144- to 147-bp fragment for *vat*, *satA*, and *vatB* (2). PCR performed with these primers resulted in a ca. 150-bp fragment for UW1965K1. A digoxigenin-labelled probe of the amplified fragment was prepared, hybridizing with a 5.5-kbp fragment of *EcoRI*-digested plasmid DNA from the transconjugant. The corresponding plasmid fragment was cloned into pUC18 and sequenced.

The resulting DNA sequence (Fig. 1) did not show significant identity with other gene sequences from GenBank on the DNA level (6). One suitable open reading frame (ORF) was found, giving rise to a putative 214-amino-acid (214-aa) pro-

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1  cggtacccegg ggatcctcta gactataatt aaaattaaat aactcaattc ggaaggtacta
      start      primor ont01
61  actctgactat acctgacgca aatgcaatct atcctaactc agccatcaaa gaggtgtctc
      M T I P D A N A I Y P N S A I K E V V F
121 ttatcaagaa cgtgatcaaa agtcccaata ttgaatttgg ggactacacc tattatgatg
      I K N V I K S P N I E Y G D Y I Y Y D D
181 acccagtaaa tcccaccgat ttgagaaac acgttaccca tcactatgaa ttcttaggcg
      P V N P T D F E K H V T H H Y E F L G D
241 acaaattaaat catcggtaaa tttgttcta tcgccagtgg cattgaattt atcatgaacc
      K L I I G K F C S I A S G I E F I M N G
301 gtgccaacca cgtaattgaaa ggtatttcga cttatccatt taatatttta ggtggcgatt
      A N M V M K G I S T Y P F N I L G G D W
361 ggcaacaata cactcctgaa ctgactgatt tgccgttgaa aggtgatact gtagtgggaa
      Q Q Y T P E L T D L P L K G D T V V G M
421 atgacgtgtg gtttgggcaa aatgtgaccg tcctaccagg cgtaaaaaa ggtgacgggt
      D V W V G E N V T V L P G V K I G D G A
481 ccattatcgg agcaaatagt gttgtaacaa aagacgtcgc tccatataca attgtcgggt
      I I G A N S V V T K D V A P Y T I V G G
541 gcaatccaat tcaactcatc ggaccaagat ttgaaccgga agttattcaa gcattagaaa
      N P I Q L I G P R F E P E V I Q A L E N
601 atctggcatg gtggaataaa gatattgaat ggataactgc taatgttctc aaactaatgc
      L A W W N K D I E W I T A N V P K L M Q
661 aaacaacacc cacacttgaa ttgataaaca gtttaattga aaatctctaa caaaaaagcc
      T T P T L E L I N S L M E K
721 gtgcaagcaa tccaaaaatg attgtttaca cggcctttac tatttagtga atccaattta
781 ttaataatag atatgatata ccagtaaaaa atacactagc cacctctggc ggtactctac
841 tcgtatatatt tatttacgac cttctgatga taaaggtcac ttccctgtcc ccagaaaaata
901 aagc

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FIG. 1. A 904-bp sequence located on the 5.5-kbp cloned fragment in pUC18 (GenBank accession no. AF139725). The ORF begins at nucleotide 63 with an ATG start codon preceding a putative ribosomal binding site (RBS) (double-underlined) at positions 50 to 57. The predicted gene sequence encodes a protein of 214 aa which shows significant homology with other streptogramin acetyltransferases (aa motifs I, II, III; see also Fig. 2). The locations of the primers *satG1* and *satG2*, specific only for the *satG* sequence, are underlined (plus strand).



FIG. 2. Alignment of amino acid sequences of acetyltransferases from staphylococci and enterococci (1-3, 9) conferring resistance to streptogramin A antibiotics. Identical residues are indicated by asterisks. Highly conserved regions in different streptogramin A acetyltransferases—motifs I, II, and III—are boldfaced. Primers satI and satII have been designed on the basis of the corresponding nucleotide sequences in motifs II and III (2).

tein. A comparison of amino acid similarities indicated rather significant homology between streptogramin acetyltransferases and the new putative acetyltransferase, designated satG (Fig. 2). Based on the sequence for *satG*, two primers, satG1 and satG2, have been designed. Preliminary results of a search for streptogramin-resistant enterococci (*E. faecium*, *E. hirae*, and *E. durans*) revealed the existence of the *satG* gene in 9 of 23 isolates from sewage, 6 of 24 isolates from broiler samples, and all 17 isolates from poultry manure. Of 62 quinupristin-dalfopristin-resistant *E. faecium* (QDREF) isolates from hospitals in Germany, 9 were positive for *satG*. The high number of *satG* QDREF isolates from poultry meat and manure may be due to selection of these bacteria by use of virginiamycin as a feed additive, and spread of the resistance via the food chain to humans is very likely. This hypothesis is being investigated.

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- G. Werner**
W. Witte
Robert Koch Institute Wernigerode Branch
Burgstrasse 37
D-38855 Wernigerode
Germany

G. Werner
W. Witte
Robert Koch Institute Wernigerode Branch
Burgstrasse 37
D-38855 Wernigerode
Germany